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PROCEEDINGS
# of the
CITRUS CHEMISTRY
AND
UTILIZATION CONFERENCE

Held at
Winter Haven, Fla.
October 5, 1966

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

FOREWORD

The Conference on Citrus Chemistry and Utilization is sponsored by the Southern Utilization Research and Development Division (SURDD) of Agricultural Research Service to acquaint representatives of the citrus industry with current developments in utilization research and to provide for an exchange of information that will benefit future research. Presentations made at this Conference report citrus research by Laboratories of the Agricultural Research Service.

This conference was held October 5, 1966, in Winter Haven, Fla., with R. A. Dennison, University of Florida, Gainesville, Fla., serving as General Chairman. The program was developed by B. H. Wojcik, Assistant Director, under the guidance of C. H. Fisher, Director, (SURDD) in cooperation with staff members, and advisers representing the citrus industry.

This report summarizes the statements of the various speakers during the conference, and gives an account of the discussions that followed. If further details are desired regarding any subject presented here, they may be obtained by communicating with the author concerned.

Underscored numbers in parentheses refer to references or literature cited at the end of the article. The figures, references, and tables are reproduced essentially as they were supplied by the writer of each paper.

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TABLE OF CONTENTS

Page

R. A. Dennison, General Chairman

SESSION I

Clarence W. DuBois, Chairman

Isolation, Characterization and Differentiation of Some Coumarins and Flavonoids in Grapefruit
J. F. Fisher and H. E. Nordby (Presented by J. F. Fisher) 1

Some Aspects of Carotenoid Pigment Formation in Desert Grapefruit
H. Yokoyama and M. J. White (Presented by H. Yokoyama) 5

Review of Analyses of "Off-Flavors" in Aged Foam-Mat Orange Crystals and Model Systems
P. E. Shaw, J. H. Tatum, and R. E. Berry (Presented by P. E. Shaw) 7

A Thin-Layer Chromatographic-Colorimetric Method for The Determination of Naringin in Grapefruit
J. F. Fisher, H. E. Nordby, and T. J. Kew (Presented by J. F. Fisher) 9

The Effect of Temperature on The Quality and Color of Texas Red Grapefruit
A. E. Purcell, R. H. Young, E. F. Schultz, Jr., and F. I. Meredith (Presented by F. I. Meredith) 13

Evaluation of WURVAC
A. I. Morgan, Jr. and J. L. Bomben (Presented by A. I. Morgan, Jr.) 14

Experiences with Bromate Method of Estimating Recoverable Oil in Citrus Juices
W. C. Scott and M. K. Veldhuis (Presented by W. C. Scott) 16

Flavoring Effects of Nootkatone in Grapefruit Juice
R. E. Berry, M. G. Moshonas, and C. J. Wagner, Jr. (Presented by M. G. Moshonas) 20

A Review of Freeze-Drying Studies at Winter Haven on Citrus Products
J. L. Froscher and R. E. Berry (Presented by R. E. Berry) 23

Recovery of Pigments from Orange Peel and Use in Enhancing Color of Orange Juice
J. F. Fisher, T. J. Kew, and H. E. Nordby (Presented by J. F. Fisher) 26

Attendance List 27

List of Citrus Publications and Patents (Sept. 1, 1965 - Aug. 31, 1966) 31

PROCEEDINGS OF THE CITRUS CHEMISTRY AND UTILIZATION CONFERENCE HELD AT WINTER HAVEN, FLA., OCTOBER 5, 1966

R. A. Dennison, General Chairman

Dr. Dennison, General Chairman, called the conference to order and introduced Dr. B. H. Wojcik who, on behalf of the Agricultural Research Service, welcomed those in attendance.

SESSION I: Clarence W. DuBois, Chairman

ISOLATION, CHARACTERIZATION AND DIFFERENTIATION OF SOME COUMARINS AND FLAVONOIDS IN GRAPEFRUIT

by
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Fruit and Vegetable Products Laboratory
Winter Haven, Fla.

(Presented by J. F. Fisher)

This investigation is part of a comprehensive study of the composition of citrus products and the relationship of composition to quality. During this work, a number of regions on thin-layer chromatoplates showed under UV light a fluorescence characteristic of coumarins.

This paper reports the isolation of nine coumarins found in Florida grapefruit. Five gallons of cold-pressed Florida grapefruit peel oil were concentrated at a ratio of 12:1 with an Arthur F. Smith rotafilm molecular still, at 60°-65° (6 m. m.), to a residue of 1,200 ml. This residue was extracted with acetonitrile. The acetonitrile was removed with a rotary evaporator at 60° (6 m. m.), affording the crude coumarin extract.

Chromatographic separations were achieved using silica gel with hexane and hexane-ethyl acetate and on 100 g. of neutral alumina (80 to 200-mesh) in a 25- X 520-m. m. column employing hexane and hexane-benzene (1:1) solution. Aliquots of the fractions were spotted on thin-layer chromatoplates and developed. The chromatoplates were examined under ultraviolet light and sprayed with $FeCl_3$-$K_3Fe(CN)_6$ reagent, which instantly detected phenolic compounds as dark blue areas. Nonphenolic psoralens were observed as light-blue areas after the application of heat. Under ultraviolet light, coumarins fluoresced violet to blue, whereas psoralens were reddish brown to yellow. Various fractions were combined according to their R_f values and fluorescence on thin-layer chromatoplates. Table 1 lists the fractions as combined off the silica gel column and the coumarins isolated.

Table 1. --Coumarins and psoralens separated on silica gel and obtained from the various hexane-ethyl acetate eluate fractions

Fractions combined	Total volume	EtOAc in eluate	Compound isolated
18-24	6.0	2.5	bergamottin
25-59	40.0	2.5	7-geranyloxy-coumarin
60-70	12.0	2.5	osthol
89-99	8.0	5.0	limettin
100-110	12.0	5.0	bergapten
128-136	9.0	7.5	5-[(3, 6-dimethyl-6-formyl-2-heptenyl)oxy]-psoralen
159-167	10.0	10.0	7-methoxy-8-(2-formyl-2-methylpropyl)-coumarin
189-190	2.5	100	bergaptol
191	1.0	100	7-[6, 7-dihydroxy-3, 7-dimethyl-2-octenyl)oxy]-coumarin

The coumarins, 7-geranyloxycoumarin and osthol and the psoralen bergaptol, all previously reported in grapefruit were identified by IR, UV, NMR, and mass spectrometry, as well as R_f values.

5-[(3, 6-dimethyl-6-formyl-2-heptenyl) oxy]-psoralen, 7-methoxy-8-(2-formyl-2-methylpropyl)-coumarin and 7-[6, 7-dihydroxy-3, 7-dimethyl-2-octenyl) oxy]-coumarin are new compounds. The last compound was found earlier in grapefruit but its structure was not elucidated until now. Bergamottin, limettin, and bergapten are known compounds but have not been previously reported in grapefruit.

Concentration of fractions 18-24, from the silica gel column, followed by further purification on the alumina column, separated a compound which fluoresced yellow under ultraviolet light. Mild acid hydrolysis afforded bergaptol, which was identified by spectroscopic means. The NMR spectrum of the compound isolated from fractions 18-24 was consistent with the structure of bergamottin. The compound was identified as bergamottin by comparing its infrared, ultraviolet, and mass spectra, as well as its R_f values, with those of authentic bergamottin.

Fractions 89-99 afforded solid material from which a blue fluorescent compound was isolated by preparative thin-layer chromatography. The compound's IR, UV, and mass spectra, as well as R_f values, were identical with those of authentic limettin.

Fractions 100-110 afforded crystals that were repeatedly recrystallized from acetone to a constant melting point of 188-189°. The admixture melting point with authentic bergapten remained unchanged. The R_f values of this compound and authentic bergapten were identical, as were their IR, UV, NMR, and mass spectra.

Fractions 128-136 furnished a compound that was observed to be pure by thin-layer chromatography in five different solvent systems (figure 1) and which on thin-layer chromatoplates, showed a positive aldehyde reaction when sprayed with 2, 4-dinitrophenylhydrazine. This reaction was not observed with nonformylated psoralens. This compound fluoresced yellow under UV light. The fluorescence excitation and emission spectra showed peaks at 348 mμ and 465 mμ, respectively.

$C_{21} H_{22} O_5$

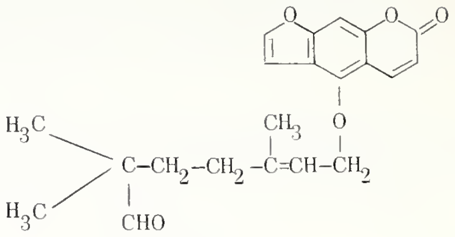

Figure 1

The compound melted from 134°-136°. It showed a negative magnesium-hydrochloric acid test for flavonoid compounds. The elemental analysis agreed with a molecular formula of $C_{21} H_{22} O_5$. The UV spectrum is characteristic of a psoralen. Addition of base did not shift the UV spectrum, indicating the absence of a free phenolic hydroxyl group. The IR spectrum displayed bands at 2, 720 and 1, 730 $cm.^{-1}$ In a compound containing only one carbonyl function these bands would be highly suggestive of an aldehyde. However, since the coumarin lactone ring also adsorbs in the region of 1, 730 $cm.^{-1}$ this band can only be used as evidence for the presence of a carbonyl group. Bands in the 1, 550 to 1, 630 $cm.^{-1}$ region are characteristic of an aromatic ring. A band at 1, 080 $cm.^{-1}$ is due to a benzofuran structure.

When this compound was warmed with aqueous methanolic KOH a yellow potassium salt was formed that relactonized on acidification. This is a reaction which is characteristic of coumarins. Mild acid hydrolysis gave 5-hydroxypsoralen, a reaction that is characteristic of an allylic ether substituent at C-5 of psoralen.

The NMR signals were in excellent agreement with the proposed structure.

The mass fragmentation pattern showed prominent ions characteristic of the fragmentation of the allylic side chain which accomplished under electron impact, thus, a parent peak or molecular weight was not obtained. Fission of this side chain α to the ether oxygen with hydrogen rearrangement gave ion m/e 202 corresponding to the 5-hydroxypsoralen fragment and ion m/e 153 corresponding to the aliphatic aldehyde fragment. The facile loss of CO, characteristic of the α-pyrone ring, gave ion m/e 174 from ion m/e 202.

Fractions 159-167 afforded a compound which after recrystallization, first from ethyl alcohol and then from a 3:1 mixture of hexane and ethyl acetate, gave fine white needles melting from 137-138°.

This compound shown in figure 2 was observed to be pure by thin-layer chromatography in five different solvent systems. On thin-layer chromatoplates, it displayed a positive aldehyde reaction when sprayed with 2, 4-dinitrophenylhydrazine. This reaction was not observed with nonformylated coumarins.

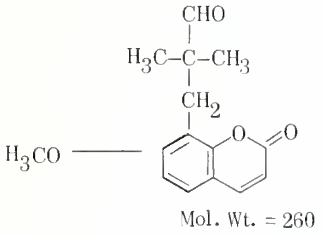

Figure 2

Under UV light it showed a purple fluorescence. The fluorescence excitation and emission spectra displayed peaks at 352 mμ and 390 mμ , respectively. The bathochromic shift observed in the emission spectrum of the previous compound relative to that seen for this one is ascribed to the furano structure of psoralens. The UV spectrum is characteristic of a coumarin and was unchanged by the addition of base. The IR spectrum disclosed bands at 2, 820, 2, 720, and 1, 720 cm.$^{-1}$ As in the case of the previous compound the band at 1, 720 cm.$^{-1}$ can only be used as evidence for the presence of a carbonyl group. A band at 1, 590 cm.$^{-1}$ is characteristic of an aromatic ring. The compound showed a negative magnesium-hydrochloric acid test for flavonoid compounds but formed a yellow potassium salt that relactonized on acidification. Under acidic hydrolytic conditions it remained unchanged.

The NMR analysis is in excellent agreement with the proposed structure.

The molecular weight as determined by mass spectrometry was 260. Other major ions were consistent with the structure as shown.

The residue from fraction 191 was recrystallized first from ethanol and then from ethyl acetate giving crystals, m. p. 124°-125° and $[\alpha]^{28}_{D} = +27°$ (figure 3). The elemental analysis agrees with a molecular formula of $C_{19} H_{24} O_5$. A hydroxyl determination showed that this compound contained two hydroxyl groups. The UV absorption spectrum is characteristic of a coumarin and is unchanged by the addition of base, indicating the absence of a free phenolic hydroxyl group. The IR absorption spectrum discloses bands at 3, 550, 3, 400, 1, 725, and 1, 615 cm.$^{-1}$ The first two bands are in the hydroxyl region, whereas the latter two are indicative of the coumarin lactone ring and an aromatic ring, respectively. A molecular weight of 332 is obtained by mass spectrometry. Figure 4 shows the partial fragmentation of this compound under electron impact.

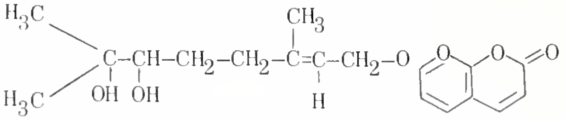

Figure 3

The NMR spectrum discloses signals that are in complete accord with the proposed structure (figure 5).

The fluorescence excitation and emission spectra show λ^{EtOH}_{ex} 352 mμ and λ^{EtOH}_{em} 390 mμ; these values are in the range obtained for similarly constituted coumarins.

The sum of the physical evidence indicates the constitution of this compound to be 7-[(6', 7'-dihydroxy-3', 7'-dimethyl-2'-octenyl)oxy]-coumarin.

This conclusion is confirmed by the following chemical evidence. The compound gives a negative magnesium-hydrochloric acid test for flavonoids. Mild acid hydrolysis gives 7-hydroxycoumarin, a reaction which is characteristic of an allylic ether substituent at C-7 of coumarin. Lead tetraacetate oxidation gives acetone and an aldehyde. This can be seen in figure 6. The aldehyde was confirmed by elemental analysis, IR, UV, NMR, and its 2, 4-DNPH derivative, as was the acetone.

The differentiation of the flavonoids naringin and its tasteless isomer naringenin-

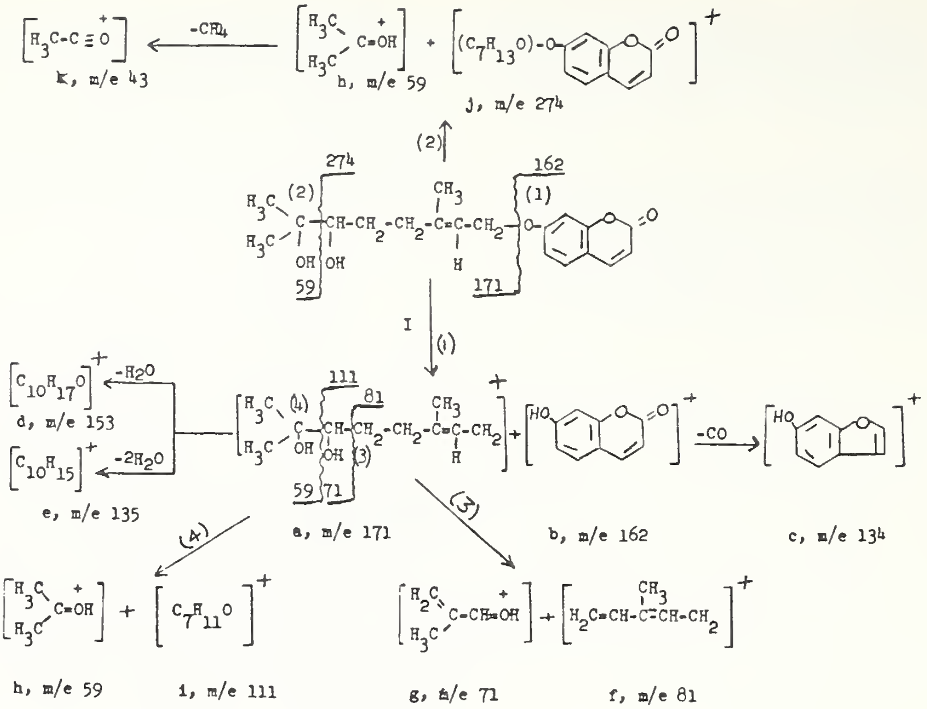

Figure 4

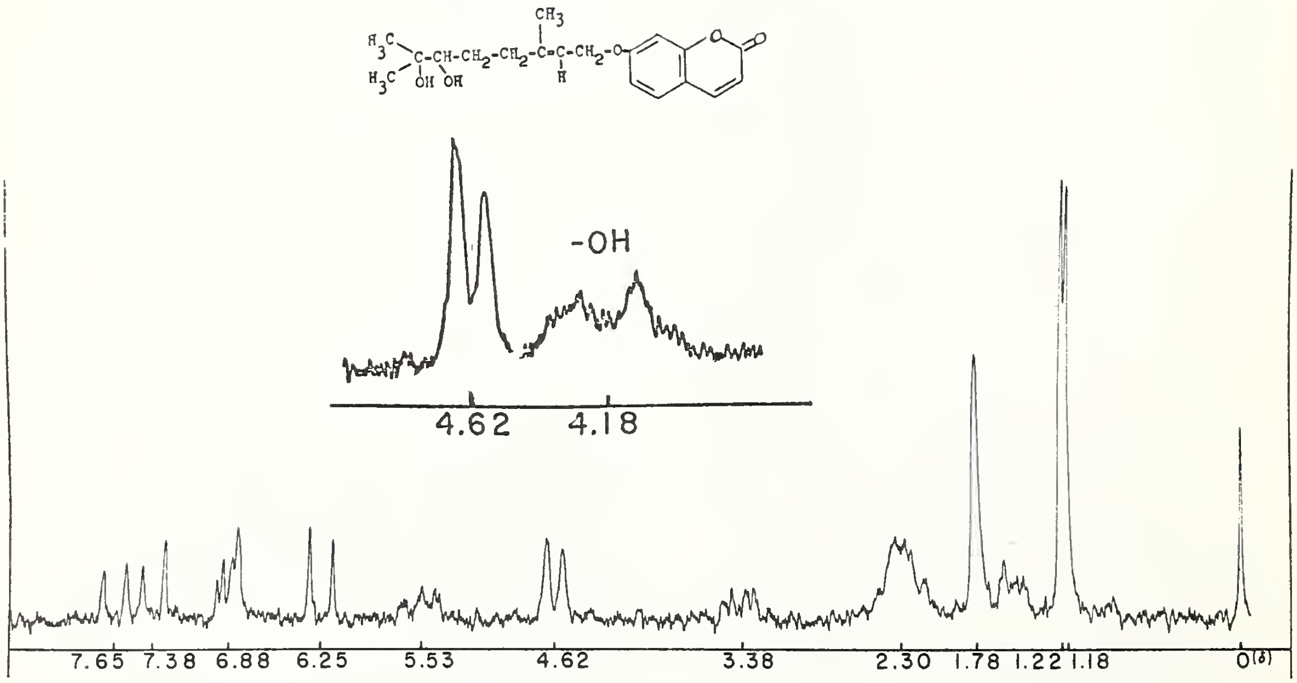

Figure 5

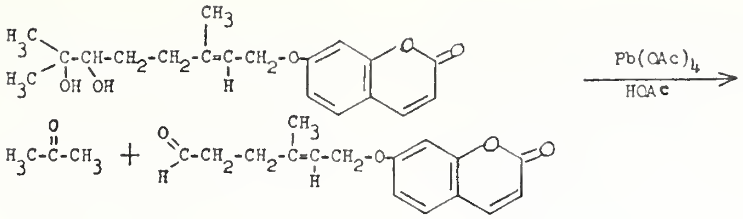

Figure 6.

7β-rutinoside by NMR has been reported by Dr. Mabry of the University of Texas. This was accomplished by preparing the trimethylsilyl ethers or the acetates of the flavonoids. We have found that it is not necessary to prepare derivatives if the free flavonoids are dissolved in pyridine and the NMR analysis conducted. Figure 7 shows the partial NMR spectrum of a 1:1 mixture of naringin (bitter) and naringenin-7β-rutinoside (tasteless) in pyridine. The absorptions (doublets) are due to their methyl protons at 1. 75 and 1. 55 p. p. m., respectively.

DISCUSSION

Question: Can you tell us which of the compounds give grapefruit its particular flavor?

J. F. Fisher: Later today we will hear a paper which will discuss this. It is not one of the compounds which we saw here. It is a compound called nootkatone.

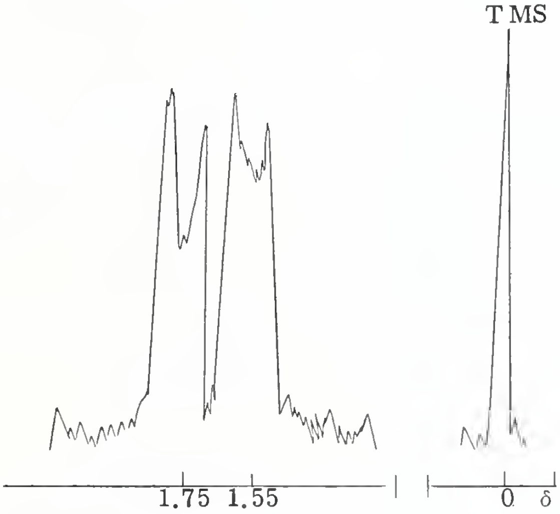

Figure 7

SOME ASPECTS OF CAROTENOID PIGMENT FORMATION IN DESERT GRAPEFRUIT

by
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Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory
Pasadena, Calif.

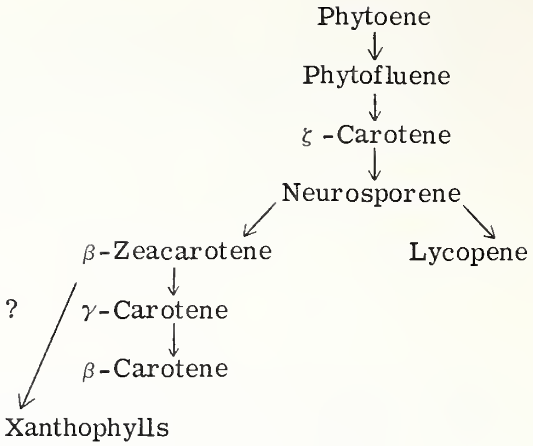
(Presented by H. Yokoyama)

The carotenoids in the peel of the Marsh seedless grapefruit were studied at various stages of maturity of the fruit.

The green peel contains the same major carotenoids as the leaves, and the colorless polysenes, phytoene and phytofluene, occur only in trace amounts in both green peel and leaves. This phenomenon appears to be invariable in all photosynthetic tissues, so that a uniform pattern of carotenoids is always found in both the green plant and the green peel. However, as the fruit ripens and its photosynthetic ability disappears, control of carotenoid synthesis is removed; and the ripe fruit exerts its own individual ability for nonphotosynthetic synthesis and accumulation of carotenoids. The distribution pattern of the carotenoids then varies with degree of maturation.

In early season fruit (at fully maturity, shortly after the disappearance of chlorophyll), phytoene is still present only in trace amounts; but a rapid buildup of phytofluene and an η-carotene-like compound has increased these two components to nearly 36% of the total carotenoids. This behavior of the η-carotene-like component of ripening fruit has been observed in a number of other citrus fruits. It is usually present in the fruit at the stage of maturity coincident with the disappearance of the green color from the flavedo, but on further ripening it disappears.

By contrast, in midseason fruit, the colorless phytoene constitutes the main constituent in the peel and accounts for nearly 51% of the total carotenoids. This accumulation of phytoene is significant. In ripening fruit, the development of the ripe color (colored carotenoid pigments) begins prior to the decrease in chlorophyll. This is the active period of carotenoid pigment formation. With the disappearance of chlorophyll (green color) and further ripening of the fruit, there is a decrease in the total colored carotenoid content of the flavedo. That is, no net synthesis of total colored carotenoids occurs. However, during this same period, phytoene accumulates rapidly in the flavedo. This suggests that with the disappearance of photosynthetic ability an inhibition of carotenogenenesis occurs that permits phytoene to accumulate, but does not allow it to be converted on to phytofluene and to colored carotenoid pigments. The nature of the inhibition or blockage remains to be investigated; but whatever its nature, it seems apparent that the blockage is the reason for the very light color of ripe desert grapefruit.



Scheme for the conversion of phytoene into fully saturated carotenes and xanthophylls.

DISCUSSION

Question: Maybe Dr. Yokoyama can explain why Florida oranges are just slightly lighter in color than California oranges.

H. Yokoyama: I think the primary reason is the temperature difference between Florida and California, where temperatures are certainly lower. It has been demonstrated in the study of tomatoes that, at temperatures above 30° C., synthesis of lycopene ceases and β-carotene is a lighter colored pigment than lycopene.

Question: Have the temperatures most effective (in influencing color formation) been isolated?

H. Yokoyama: I don't think any work has been done other than that which Mr. Meredith will report today on grapefruit. No work has been done on oranges to my knowledge. Undoubtedly other factors than temperature are involved, such as sunlight. It is well known that pyracantha berries exposed to direct sunlight are yellow, and in the shade are red. Sunlight favors trans-cis isomerization, cis-lycopene being formed in the sun and trans-lycopene in the shade.

Question: Do you suppose maturity of grapefruit could be described by its phytoene content?

H. Yokoyama: Studies are still tentative with respect to what you have reference to, but we might be able to correlate the buildup of phytoene with maturity. Right after the disappearance of chlorophyll, and the fruit has obtained its deep yellow color, it is still very low in phytoene. But you can correlate this with the buildup of phytofluene coincidentally with the disappearance of chlorophyll. You have to go later on into the season before you can see it. You certainly can correlate maturity with color in the midseason fruit.

Question: Can you give us an idea as to the carotenoid content of the peel, say on a dry weight basis?

H. Yokoyama: We do not have the percentage based on dry weight, but we do have it on a wet basis. It is very low; in green peel it is about 11-12 mg. /Kg. of wet peel; in early season (yellow color) about 2.5 mg. /Kg.

Dr. Yokoyama goes on to call attention to the high proportion of albedo present, so concentration would be much higher in the flavedo.

REVIEW OF ANALYSES OF "OFF-FLAVORS" IN AGED FOAM-MAT ORANGE CRYSTALS AND MODEL SYSTEMS

by
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Fruit and Vegetable Products Laboratory
Winter Haven, Fla.

(Presented by P. E. Shaw)

Foam-mat dried orange crystals of good initial quality develop detectable changes in flavor after 2 to 3 weeks at 85° F. when compared to controls stored at 0°. Grapefruit crystals, on the other hand, can be stored for 12 weeks or longer at 85° before a detectable change in flavor occurs. At higher temperatures and longer periods of storage both develop characteristic objectionable flavors. We have undertaken a study to determine the nature of the changes occurring in orange crystals during storage. We have also examined grapefruit crystals after storage to compare the changes that occur with those in orange crystals.

In an initial publication, we described the qualitative detection of 5-hydroxymethylfurfural in stored orange crystals by thin layer chromatography. This compound has no appreciable odor, and so is unlikely to be responsible for changes in flavor that develop. However, it did appear to be indicative of other changes which had occurred. It was previously known that 5-hydroxymethylfurfural could be produced from fructose upon heating under acidic conditions. We heated a model system of pure fructose in water, which had been adjusted with citric acid, to the approximate pH of orange juice. The products we isolated from this model reaction were furfural, acetylformoin, 5-methylfurfural, isomaltol, 2-hydroxyacetylfuran, 2-hydroxyacetylfuran formate, 4-hydroxy-2-hydroxymethyl-3(2H)-furanone, 5-hydroxymethylfurfural, formic acid, acetic acid, and levulinic acid.

We had previously worked out an extraction procedure with "off-flavor" orange crystals that afforded a fraction showing, by thin-layer chromatography, some of the same products that were produced in the fructose degradation. Our extraction procedure involved separation of the carotenoid, flavonoid, and furan derivatives from pectins and sugars by ether extraction and then separation of the water-soluble flavonoids and furans from the carotenoids by washing. Vacuum distillation separated the furanoid components from the less-volatile flavonoids. The distillate containing the furans was separated by gas chromatography.

From the furan fraction we identified all of the compounds that we had found in the fructose degradation above except for isomaltol, hydroxyacetylfuran formate, and formic acid. In addition, we identified several other components of this fraction from "off-flavor" orange crystals which could not be detected in fresh orange crystals by the same analytical procedure.

Some of the products that we have identified possess odors that would be considered objectionable if detected in orange juice. Thus, acetylformoin and levulinic acid have strong caramel-like odors, and methylcyclopentenolone has such a strong maple-like odor that it is used commercially in food products to give them that flavor. The odor of 4-hydroxy-2-hydroxymethyl-3(2H)-furanone is more like charred sugar. Furfuryl alcohol and γ-butyrolactone have odors resembling that of rancid fat. Five other components all have unpleasant odors similar to that of furfural. These materials are all present in such small amounts that their individual contributions to the "off-flavor" that develops has not been determined. Ten of these fractions, when combined and added to control orange juice, gave it a flavor very similar to that encountered from stored orange crystals.

Analysis of grapefruit and orange crystals by our procedure revealed that samples of both products which had developed "off-flavors" contained the same furan derivatives in about the same quantities.

Limited success has been achieved so far in attempts to slow down the browning reaction that causes "off-flavors" to develop upon storage. Thus, decreasing the moisture content of orange crystals from 3 to 1 percent resulted in a slightly longer storage life, as did addition of bisulfite. Reducing moisture below 1 percent had little further effect.

DISCUSSION

Question: What was the moisture level of these samples when they were stored, and were they stored with desiccant bags?

P. E. Shaw: The best samples were at 1 percent moisture. Lowering the moisture below that by use of desiccant didn't increase the storage life.

Question: Were these samples stored in cans or bags, and what was the atmosphere of storage?

P. E. Shaw: They were stored in cans, sometimes under CO_2. Taste tests showed that the small amount of air you would have under normal storage would not matter much.

Question: You didn't consider that pectin or citric acid might break down?

P. E. Shaw: We considered that, but we wanted a system that would give us the highest yield of these furans, and used fructose because it was easiest. Certainly, sucrose can give the same thing, but it would take a little longer, and of course glucose can do the same thing.

Question: Ascorbic acid?

P. E. Shaw: I do not see it as likely. It is possible, but I think the sugars are the most likely sources. There is nothing to keep Vitamin C from breaking down the same way.

Question: Have you followed the breakdown of amino acids?

P. E. Shaw: No, but we hope to.

Question: Did your model system undergo browning?

P. E. Shaw: Yes, but there were no insoluble brown polymers formed in the heating period we used. It does turn quite dark. Upon adding glycine, it turns brown at about the same rate.

A THIN-LAYER CHROMATOGRAPHIC-COLORIMETRIC METHOD FOR THE DETERMINATION OF NARINGIN IN GRAPEFRUIT

by
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Southern Utilization Research and Development Division
Fruit and Vegetable Products Laboratory
Winter Haven, Fla.

(Presented by J. F. Fisher)

The principal flavanone glycoside in grapefruit is naringin, which is the 7β-neohesperidoside of naringenin. This is the compound primarily responsible for the bitterness of grapefruit. Its isomer, the 7β-rutinoside of naringenin also present in grapefruit, is tasteless. Other compounds present in grapefruit such as limonin and poncirin are secondary contributors to bitterness.

Because bitterness is frequently cited as the principal deterrent to the profitable marketing of grapefruit products, several procedures have been reported for the determination of naringin. The Davis test, when applied to whole grapefruit juice, suffers from the disadvantage of not being able to differentiate between naringin and its tasteless isomer. Therefore, it is not a reliable measure of naringin bitterness. The chromatographic-fluorometric method reported by Hagen and coworkers appears to be reliable. However, it is somewhat involved for routine determination of grapefruit bitterness.

This paper reports a relatively simplified quantitative determination of naringin employing a modification of both the thin-layer chromatographic system reported by Hagen and coworkers, and Mizelle and coworkers, and the Davis test.

The grapefruit used throughout this work was the Florida Duncan variety, harvested November 1965 through January 1966.

The procedure is as follows:

1. A 100-ml. sample consisting of both grapefruit juice and sacs was blended for one minute in a Waring blendor and then filtered through glass wool.

2. A 50-ml. sample of the filtrate was placed in a 250-ml. round-bottomed flask and concentrated with a rotary vacuum evaporator and filter pump (aspirator) at 45° to a viscous residue.

3. This residue was readily transferred to a 15-ml. centrifuge tube with a disposable pipette, 15 cm. long. The volume of residue in most cases was found to be about 8 ml. The flask was rinsed with just enough water to adjust the volume in the centrifuge tube to 10 ml. The flask was then rerinsed with several portions of methyl alcohol to give 15 ml. of extract with some suspended solids in the centrifuge tube.

4. The contents of the centrifuge tube were well mixed and centrifuged at top speed for 5 min. in an International Clinical centrifuge model CL.

5. A 15 μl. sample of the supernatant extract from step 4 was streaked with a disposable micro-pipette accurate to within 1 percent or less, along a 16-cm. long pencil line drawn on a firm, nonflaky polyamide adsorbent contained on a 20- x 20-cm. glass plate. The firm, nonflaky polyamide adsorbent was prepared as follows: A mixture of 0.8 g. of rice starch, 0.4 g. of silica gel (Fisher No. 1 impalpable powder) and 9 ml. of water in a covered 20 ml. beaker was heated for 40 min. on a steam bath with occasional stirring. Water (1-2 ml.) was added during the heating as needed to prevent caking. This mixture was rinsed with 3 ml. of water into a 100 ml. beaker containing 5.5 g. of Woelm polyamide powder and 35-40 ml. of methanol. This mixture was stirred and then blended in a Waring blendor microcup for 3 min. The resulting mixture was spread as a 250 μ thick layer on 20- x 20-cm. glass plates and allowed to dry 2 hr. at room temperature before use. The pencil line does not destroy the layer and the firmness of this adsorbent layer helps to insure against mechanical loss.

6. The chromatoplate was developed twice in the 5:2, nitromethanemethyl alcohol system reported by Hagen and coworkers. By developing the chromatoplate twice a better separation was achieved between naringin and naringenin-7β-rutinoside. Each development required about 45 min. at 25° in a rectangular thin-layer chromatographic tank with a filter paper liner.

7. After development, the plate was allowed to dry and lightly sprayed with 1 percent $AlCl_3$ in ethyl alcohol and exposed to UV light (3, 660 Å). Both naringin and its tasteless isomer appear as bright yellow fluorescent bands.

8. The naringin band located by its yellow fluorescence in step 7 and also by the use of an authentic naringin marker, R_f 0. 31, was outlined with a pencil and the area was lightly sprayed with water. The naringenin-7β-rutinoside has an R_f value of 0. 38. The damp polyamide-naringin area was scraped from the plate and placed in a 10- x 75-mm. test tube (a small soft brush was used for brushing the scraped area clean). The water spray prevents the formation of an electrostatic charge, which could cause a loss while scraping.

9. Into the test tube containing the scrapings, 1. 50 ml. of the test reagent was added from a burette. The test reagent consisted of 125 ml. methyl alcohol, 112 ml. diethylene glycol, 13 ml. water and 5 ml. of 4N aqueous sodium hydroxide. The scrapings and reagent were well mixed by syringing with a disposable pipette. This procedure elutes the naringin from the polyamide and initiates the formation of a yellow color which should be allowed to develop for 10 min. before reading in a colorimeter.

10. The test tube was stoppered and centrifuged at top speed for 3 min. The clear supernatant was removed with a disposable pipette and transferred to a clean 10- x 75-mm. test tube.

11. The intensity of color was determined with an Evelyn photoelectric colorimeter using a blue filter (420 mµ) against a blank carried through the procedure from step 6. An adapter, to accommodate matched test tubes 10- x 75-mm., was placed in the tube holder. A minimum volume of 0. 8 ml. of sample was required. Any colorimeter that can handle micro samples could be used.

12. The galvanometer reading, T (percent transmittance), was converted to A (absorbancy) by ($A = 2 - \log T$), from which the micrograms of naringin were determined from a standard curve. The standard curve was prepared as follows: A standard solution of chromatographically pure naringin in methyl alcohol-water (1:2, $^{v}/v$) containing 1 µg. naringin per microliter was prepared. Nine light pencil lines, each 16 cm. long, were drawn on a TLC plate composed of the same thin-layer adsorbent used in the procedure (step 5). Aliquots from 5 to 40 microliters in 5 microliter increments of the standard naringin sample were applied with disposable micro-pipettes along the entire length of eight of the pencil lines. The first line contained 5 µg. of naringin, the second 10 µg., and so on through 40 µg. along the eight line. The ninth line was used in preparing the blank. The plate was lightly sprayed with $AlCl_3$ and the procedure starting with step 8 was carried through step 11.

The galvanometer reading was converted to absorbancy as in step 12 of the procedure and plotted along the ordinate versus the corresponding µg. of naringin along the abscissa. Standard curves were also prepared by developing the standard naringin samples in the nitromethane-methyl alcohol solvent system and scraping from the plates in the same manner as the grapefruit samples, as well as. by the direct addition of the various standard naringin samples to the test reagents both with and without the addition of $AlCl_3$ and polyamide. In all cases the standard curves were essentially superimposable and obeyed Beer's law throughout the 5 µg. to 40 µg. range studied.

The precision of the method can be seen in the first table which shows the results of determinations on five 50 ml. aliquots from a 500 ml. sample of grapefruit juice carried through the procedure by different investigators. Also, a series of seven aliquots (values not shown) from a different sample of grapefruit extract gave a mean value of 10. 4 µg. of naringin per 15 microliters of extract. The percent standard deviation was 1. 6.

The accuracy of the procedure was tested by investigating the percent recovery of

Table 1.--Precision of Method: Determination of two flavanone glycosides in five identical samples of grapefruit juice by different investigators

Sample No.	μg. of flavanone glycosides/15 naringin	Microliters of extract naringenin-7β-rutinoside
1	15.3	5.4
2	15.4	5.3
3	15.4	5.3
4	15.6	5.4
5	15.7	5.5
Mean	15.5	5.4

Table 3.--Concentration of naringin in the juice of three different varieties of fresh grapefruit and a commercial canned grapefruit juice

Variety	Grams per 100 ml. juice
Duncan	0.031
Mott [1]	trace
Marsh seedless	0.022
Canned juice	0.033

[1] The Mott grapefruit matures earlier than the Duncan or Marsh seedless and is, in general, considerably less bitter.

various amounts of naringin added to a "base" sample of grapefruit juice found in the precision studies to contain 10.4 μg. of naringin per 15 microliters of extract. The enriched samples were carried through the procedure. Table 2 shows the recovery of added naringin.

Experiments were conducted to determine the concentration of naringin in three different varieties of fresh grapefruit and in a commercial canned grapefruit juice. The samples were carried through the procedure and the results are shown in table 3. These results, expressed as grams per 100 ml. of juice, were obtained by multiplying micrograms per 15 ml. of extract by 0.002. These results are presented to illustrate the use of the method and are too few to indicate trends.

This analytical method for naringin can be performed on a routine basis in both research and plant laboratories. The use of both juice and sacs in step 1 was to obtain a realistic commercial juice sample. The sample sizes in steps 1 and 2 are arbitrary. The addition of methyl alcohol (acetone also works well) in step 3 coagulates the suspended solids, thereby aiding their separation by centrifugation. The precipitated solids and any residue remaining in the flask after rinsing in step 3 were examined by sodium borohydride-hydrochloric acid; magnesium-hydrochloric acid and thin-layer chromatography. All the solid residues were virtually free of flavonoid material. The addition of methyl alcohol also affords a supernatant which has better streaking qualities such as low viscosity and higher volatility than juice alone.

Table 2.--Accuracy of Method: recovery of naringin added to a "base" sample of grapefruit extract

Sample No.[1]	Naringin	Naringin found [2]	Added naringin recovered	Recovery
	μg.	μg.	μg.	Pct.
1 [3]	0	10.4	---	---
2	5	15.7	5.3	106.0
3	10	20.3	9.9	99.0
4	15	25.4	15.0	100.0
5	20	30.5	20.1	100.5
6	25	35.1	24.7	98.8
7	30	40.0	29.6	98.6
8	35	45.5	35.1	100.2

[1] Samples 1-8 contained an average of 10.4 μg of naringin in addition to the known amounts of added naringin.

[2] Average of triplicate determinations.

[3] "Base" sample.

The sample in step 5 should be applied as a smooth, even streak. This will aid in obtaining a clean separation of naringin from naringenin-7β-rutinoside. With care and experience, one can effect a clean separation with only one development. The R_f values are 0.31 for naringin and 0.38 for naringenin-7β-rutinoside. However, two developments are recommended.

Various experiments were conducted in which both the naringin area and the naringenin-7β-rutinoside area were removed and separately rechromatographed to see if either area contained any of the corresponding isomer. The separation was adequate in all cases where the application and removal of sample had been properly performed and overloading had not occurred.

The diethylene glycol in the test reagent enhances the intensity of the yellow color over that seen in methyl alcohol. Diethylene glycol was the most effective of several glycols tested for this color enhancement. The ratio of diethylene glycol to methyl alcohol in the test reagent was chosen to afford the best compromise between color enhancement and viscosity. Clarification of the sample in step 10 is partially dependent upon a system of low viscosity.

The yellow color, which is due to the naringin chalcone formed by the action of sodium hydroxide on naringin, is fully developed after 10 min. and is stable for at least 20 hr. The adsorbent system was shown not to develop color with the test reagent.

This accurate and relatively rapid method for naringin would be applicable in investigations where the amounts of naringin and naringenin-7β-rutinoside are to be compared and in correlation studies for bitterness.

DISCUSSION

Question: How long did you say this method took?

J. F. Fisher: Three to five hours, depending on experience and skill.

Question: Have you established a constant relationship between naringin and the nonbitter glucosides? Can you apply a factor to the Davis test? The time required for your test is too long for lab control.

J. F. Fisher: The Davis method will not accurately determine bitterness, as it measures all flavonoids. You can separate flavonoids by TLC and then apply the modified Davis technique described here to measure only the bitter flavonoid. It is not likely that the time for a determination can be reduced, since a single development requires one hour in the tank. Multiple determinations can be run in the same 3-5 hour period required for a single determination.

Question: Is there a conversion factor for converting the Davis test to bitterness?

J. F. Fisher: This factor may exist, I do not have it.

Question: Aren't total glycosides rather constant throughout the life of the fruit with naringin being converted to nonbitter glycosides? During maturity?

J. F. Fisher: We are presently trying to compile data which will throw some light on this. The conversion of naringin to nonbitter naringenin-7β-rutinoside was proposed on very meager data. Dr. Wender, University of Oklahoma, published an article in Food Science which does not support nor disprove this thought. We are trying to obtain more definitive information with the procedure described here. We are investigating both leaves and fruit to see what ratios exist between naringin and naringenin-7β-rutinoside versus time. We hope this information will help indicate whether or not there is conversion.

THE EFFECT OF TEMPERATURE ON THE QUALITY AND COLOR OF TEXAS RED GRAPEFRUIT

by
A. E. Purcell[1/], R. H. Young[2/], E. F. Schultz, Jr.[3/], and F. I. Meredith[4/]

(Presented by F. I. Meredith)

From previous work at the Weslaco Laboratory it was found that the maximum concentration of lycopene occurs in Texas red grapefruit between August 15 and September 10. It was believed that an environmental factor was involved in the decline of lycopene. Weather records showed that the first cool nights occurred during this time. Temperature decline was suspected to be a factor initiating the loss of lycopene in the fruit.

The experiment involved two seasons' work. Small potted grapefruit trees with two to eight fruit were used in the first season. Small grapefruit seedlings with 1-inch-diameter grapefruit grafted on were used in the second season in place of the potted trees.

The potted trees were placed in two environmental chambers. The temperature of chamber No. 1 was 95° F. during the day and 85° at night. The second chamber was held at 60° during the day and 40° at night. The day length was 8 hours and the night length was 16 hours. Weekly samples of fruit were taken from trees in both chambers.

In the second season's work the grafted seedlings were sorted into three groups containing fruit of equal sizes. The individual fruit were numbered from 1 to 100. Group 1 was placed into a chamber with 95° F. day and 85° night temperature. Group 2 was placed into a chamber with 70° day and 60° night temperature. Group 3 was placed in full sunlight out of doors and was comparable in location to the trees in a grove. The grafted seedlings in groups 1, 2, and 3 were held under these conditions for 139 days until November 22. Fruit samples were taken every two weeks. Fruit samples were weighed, peeled, weighed again, and blended for analyses. Aliquots of the blended samples were taken for carotene-lycopene, Brix, acid, and naringin determinations. Color and peel thickness was measured on the peel of the fruit.

The fruit in the 95°/85° F. chamber maintained a dark green peel color, whereas the fruit in the 70°/60° and 60°/40° chambers lost its green color and turned a lemon yellow. The peel thickness decreased in all the treatments with the 95°/85° treatment having the largest decrease.

The lycopene content of the fruit increased in the 95°/85° treatment. Within 2 weeks after being placed in the chambers, the lycopene content of the fruit from the 70°/60° and 60°/40° chambers decreased. The Brix remained constant in the fruit from the 95°/85° chamber and the 70°/60° chamber. Brix increased in the fruit from the 60°/40° chamber. The acid increased in the 60°/40° treatment and decreased in the other treatments. The Brix/acid ratio increased in all of the temperature treatments. The naringin content remained constant through all of the various temperature treatments.

From the results of two seasons' studies it is concluded that declining environmental temperature in the fall is primarily responsible for loss of lycopene in Texas Ruby Red grapefruit.

DISCUSSION

Question: Is the color in Texas red and pink grapefruit strictly lycopene?

F. I. Meredith: Predominantly.

Question: You spoke of grafted stock - how was this done?

[1/] U.S. Food Crops Laboratory (Raleigh, N.C.), Southern Utilization Research and Development Division, ARS, USDA.

[2/] U.S. Fruit, Vegetable, Soils and Water Laboratory (Weslaco, Tex.), Crops Research Division, ARS, USDA.

[3/] Biometrical Services (Beltsville, Md.), ARS, USDA.

[4/] U.S. Fruit and Vegetable Products Laboratory (Weslaco, Tex.), Southern Utilization Research and Development Division, ARS, USDA.

F. I. Meredith: We cut fruit at a size of about 1-inch diameter with 2-inch stems, and grafted them onto small seedlings. This allowed us to have a large number of fruit with the same genetic background, as all the fruit came from only five trees. It also allowed placing a large number of fruit into these small climate chambers, and permitted uniformity of age and size of fruit.

Question: Is there a distinct difference in appearance of juice, with chemically determined variations in lycopene content?

F. I. Meredith: Yes. You can see a big difference after three samplings (6 weeks) at the 2-week treatment times, and after 3 weeks at the one-week time in the 60-40 chamber. The extracted material was definitely lighter, and you could even see a change in the blended juice itself before extractions.

Question: Could you see the difference between 2 and 3 mg. of lycopene by observing the cut fruit?

F. I. Meredith: Yes.

EVALUATION OF WURVAC

by
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(Presented by A. I. Morgan, Jr.)

The recovery of aroma from food products by most of the methods in commercial use today requires boiling at atmospheric pressure. For some products, such as citrus juices, this high temperature heat damages the product.

The WURVAC process (figure 1) for recovering the volatile aroma fraction of food products uses a vacuum to obtain the desirable low temperature and a unique gas compression technique to absorb the volatile materials in any suitable solvent.

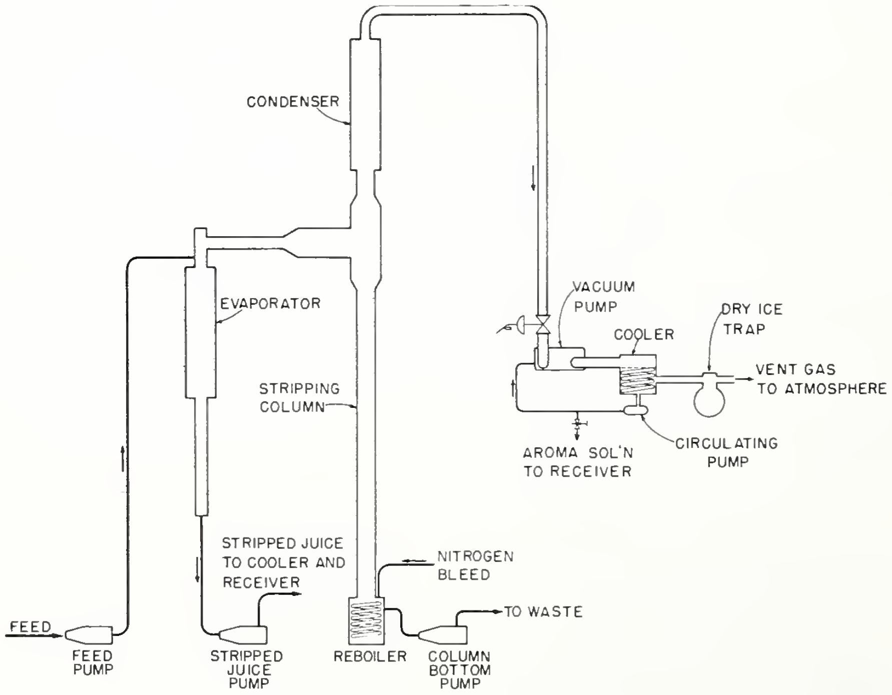

Figure 1.--Schematic diagram of WURVAC Process.

A pilot plant WURVAC unit was built in our laboratory. The feed juice (120 lb./hr.) was stripped of volatiles at temperatures between 120° and 180° F. The vapor was fed to a packed stripping column where nitrogen, bled into the vacuum system, stripped the volatiles immediately from the boiling condensate. The volatiles were then absorbed in a liquid-sealed vacuum pump where the nitrogen carrying the volatiles was compressed and intimately mixed with the circulating sealant, double-distilled water. By this process aroma solutions of 150- to 200-fold (volume of aroma solution/volume of feed juice) were made.

With the use of gas-liquid chromatography of headspace vapors, the effects of changes in operating conditions, such as temperature of evaporation, degree of concentration, reboil rate, and nitrogen bleed rate, were measured. Oil and chemical oxygen demand (COD) analyses provided additional means of evaluating the operating conditions. In the case of orange juice, evaporation of 20 percent of the feed at 180° F. gave conditions in which 90 percent of the volatiles were removed, and at the same time the juice was pasteurized. Neither the stripped juice nor the aroma solution showed any detectable damage under these conditions.

Concentrates were fortified with aroma solutions made by the WURVAC process. These concentrates were compared organoleptically and chromatographically with concentrates containing pasteurized, single-strength "cutback juice." A panel of 15 trained judges was used to evaluate the different treatments by triangle test. Reconstituted juices were judged for both aroma and flavor differences under completely independent conditions. Some of the samples were also ranked on the basis of most to least like fresh orange juice aroma. All evaluations were conducted in individual booths equipped with dim lights to eliminate influence from any possible color or appearance difference between samples. The booths were maintained at 70° ± 2° F. with a constant flow of odor free air. First, samples were presented in glass covered ruby red cups, and the panel was instructed to judge on the basis of aroma alone. After completing this evaluation the judges were presented with the same samples in 4-oz. paper cups for flavor comparison. Order of presentation was randomized and each treatment appeared as the odd sample an equal number of times. After a decision was made on the odd sample in each triangle, the judges were also asked to indicate which sample or samples had the fresher orange aroma or flavor. Each comparison for flavor and aroma was repeated twice, giving a total of 30 judgments.

Those products containing the aroma solutions were judged as being more like fresh orange juice. With the use of these aroma solutions and orange oil it becomes possible to make high quality orange concentrates of 55° Brix or more containing all the original aroma constituents, instead of a 45° Brix "cutback" concentrate containing only about 8 percent of the water-soluble aroma constituents.

DISCUSSION

Question: Have you applied for a patent?

A. I. Morgan, Jr.: Yes, a public patent of course.

Question: Dr. W. R. Roy, Minute Maid Company. The number of the Minute Maid process is 3,248,233 issued on 4-26-66

A. I. Morgan, Jr.: Thank you.

Question: Can you explain why you did not reflux vapors in your packed column? Does it interfere with the quality of the product?

A. I. Morgan, Jr.: We used 100 percent reflux. All of the liquid which is removed from the fruit is condensed in the refrigerated condenser, drains back through the packed column. We do not want any liquid from the juice in the aroma solution--the aroma is completely transferred to the solvent (water) in the Nash pump.

Question: Instead of bleeding in nitrogen, could you use petroleum ether to supply a sweeping gas? The petroleum ether perhaps could be recycled.

A. I. Morgan, Jr.: It ought to bring out some of the heavier things, in which the aroma solution is weak. You will notice the score did not really get very high until we put oil back in. Your suggestion might be good in that respect.

Question: You use water in the Nash pump. Is your aroma in oil emulsion or in water solution?

A. I. Morgan, Jr.: It is completely clear. Even when completely loaded, no cloud is apparent. Sometimes we get a cloud with apple juice, but not with orange.

Question: You are bound to get some oil over. Do you decant it, or is it emulsified?

A. I. Morgan, Jr.: This is not a good way to recover oil. We do get some oil, but the little bit that comes over is dispersed so well it does not form cloud. The use of ethanol as sealant in the Nash pump will take up a lot of oil, and develop some color.

EXPERIENCES WITH BROMATE METHOD OF ESTIMATING RECOVERABLE OIL IN CITRUS JUICES

by
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(Presented by W. C. Scott)

Introduction

Subsequent to the first public description of the bromate method at the 1965 Conference, a number of industry research and control laboratory supervisors obtained the necessary equipment and supplies to begin critical evaluation of it. Naturally, many modifications of the method were tried, some of which resulted in slight decreases in precision In April 1966, questionnaires were mailed to 25 research or control laboratories asking for an accounting of experience with the method. Replies were received from 17, two of whom had not tried the method. Replies from the other 15 are the principal source of information for this paper.

Review of Method

For the benefit of those who may not be familiar with the method, the essentials are as follows: To 25 ml. of citrus juice in a 500-ml. boiling flask, add 25-ml. of 2-propanol. Attach a specially designed foam-trap connecting tube and coil condenser and distill approximately 30 ml. of condensate into a 150-ml. beaker. To the beaker add 10 ml. diluted HCl and one drop of methyl orange indicator, then titrate with 0.025N potassium bromide-bromate solution to the disappearance of color. Multiply milliliter of titrant by 0.004 to obtain percentage of recoverable oil in the sample.

A detailed description of the apparatus and method, with supporting data, was published in the June issue of the Journal of the Association of Official Analytical Chemists.

Before discussing results of the survey we would like to review some individual experiences Informal reports have been received from time to time that, with certain types of juice products, very large differences were found between recoveries by the bromate and Clevenger methods. In each instance in which portions of these juices were made available to us, we ran comparative tests and obtained differences no greater than 0.005 percent oil. We have suggested that, when such wide differences were found, the residual juice from the Clevenger determination be tested for oil by the bromate method. Table 1 illustrates results of this type of investigation. The first three samples, tested in our laboratory in duplicate, were from a laboratory that had reported recovering twice as much oil by the bromate as by the Clevenger method. We found appreciable differences between the two methods, but they were fairly constant at three oil levels, therefore the factorial difference of two was not substantiated.

Results of examination of another sample, tested in triplicate in an industry laboratory, are also shown in this table by permission. Discrepancies between the methods were

Table 1. --Comparison of oil recoveries by bromate and Clevenger methods

PERCENT RECOVERABLE OIL

Bromate method (A)	Clevenger method (B)	Difference (A-B)	Pot. & trap residue (C)	Net diff. (A)-(B+C)
0. 0082	0. 0047	0. 004	0. 0014	0. 002
. 0082	. 0041	. 004	. 0007	. 003
. 0128	. 0083	. 005	. 0014	. 003
. 0130	. 0100	. 003	. 0014	. 002
. 0154	. 0120	. 003	. 0013	. 002
. 0156	. 0133	. 002	. 0012	. 001
. 0418	. 027	. 015	. 013	. 002
. 0425	. 038	. 005	. 007	. 002
. 0425	. 024	. 019	. 017	. 002

quite wide in two of the replications as shown in column three. When the residue was tested by the bromate method, it was clearly shown that Clevenger recoveries were inconsistent as well as incomplete.

The approximately 0. 001 percent oil found in the residues from our Clevenger tests is probably a water-soluble fraction of the oil not recoverable in the trap. The water-soluble, bromine-reactive material found in the trap is not shown separately on the table because it was consistently found to be about 0. 0002 percent of the 1, 500-ml. sample. When the oil contents of pot and trap residues were added to the Clevenger values, their differences from the bromate values were fairly constant as shown in the last column.

Results of Survey

Equipment Modifications

Thirteen of the 15 respondents used the recommended glassware, with such minor variations as flat-bottomed flasks or automatic dispensing and titrating burettes. The two others used standard diacetyl apparatus without modification (1, 000-ml. flask, standard taper connecting tube without trap, and a 300-mm. condenser). One laboratory used one set-up of recommended glassware and one diacetyl set-up.

Further results of the survey are summarized in table 2.

Comparison with Clevenger Method

Although the bromate method always gave higher results, eleven respondents observed no correlation between values. Laboratories designated as 2, 7, and 13 expressed a factorial difference, with Clevenger recoveries running from 74 to 89 percent of those by the bromate method. Laboratory 13 summarized data from more than 75 comparative tests as follows: with four inspectors running Clevengers and two laboratory technicians running bromate titrations on reconstituted FCOJ, the bromate method gave higher recoveries by an average of 0. 003 percent. This agrees with data compiled in our Laboratory during development of the new method.

Comparison with Other Rapid Methods

Seven of the 15 respondents were either not using a short method or had not made comparative tests Laboratory 2 reported 12. 5 percent higher recovery by the bromate method, and four others reported only that the bromate have higher recoveries Laboratories 5 and 11 reported equal precision between the two methods, and laboratory 12 reported greater precision with the turbidimetric method (this laboratory used diacetyl equipment only for the bromate test). All of the eight who made comparisons reported that they preferred the bromate method because of its reliability and its extended range of usefulness (at least ten-fold).

Table 2. --Summarized experience of 15 industry laboratories evaluating the bromate method

Lab. No.	Comparison of methods: Clevenger	Comparison of methods: Quick oil	Error in recov. of added oil	Variations in percent oil recov. by- 1 Oper.	Variations in percent oil recov. by- Sev. Oper.
	Pct.	Pct.			
1	nc	+12. 5	4	0. 0006	0. 0010
2	. 89B		±1	. 0001	. 0010
3	nc		0	. 0002	. 0004
4	nc			. 0010	. 0050
5	nc	iden.		VG	VG
6	nc	+	0. 2	. 0001	. 0002
7	. 74B	+	1-2	VG	VG
8	nc	+	0	VG	VG
9	nc		5	. 0008	
10	nc	+	VG		
11	nc	iden.	±1	. 0001	. 0001
12	nc	-	-12 +20	. 0006	
13	-. 003			. 0010	. 0010
14	nc		±2	VG	VG
15	. 80B		5	VG	. 0100

Accuracy in Recovery of Added Oil

Of the 12 respondents reporting tests on the recovery of added freshly distilled orange oil, eight had obtained recoveries within ±2 percent of the amount added (e. g. , . 0200 ±. 0004), two reported 95 percent recovery and Laboratory 12 reported about 88 percent recovery at 0. 160 percent added oil and 120 percent recovery at 0. 00125 percent added oil. This low recovery at high oil concentration is emphasized to illustrate what can happen when operating conditions vary widely from those recommended. In this case standard diacetyl equipment was used, and 75-ml. of water were added to the 1, 000-ml. boiling flask in addition to the recommended 25-ml. each of sample and 2-propanol. Apparently, considerably more than the recommended 30-ml. of distillate was obtained, as reference was made to large quantities of water possibly contributing to an obscure endpoint. These deviations from recommended practice may be responsible for erratic and/or low recoveries from high oil samples. The excess recovery at the 0. 00125 percent oil level illustrates that precision of the method does have limits: 120 percent recovery gave a reading of 0. 0015 percent oil, an error which we do not consider detrimental to the usefulness of the method.

Precision in Replications by One Operator

No laboratory reported variations in replicate determinations by a single operator to exceed 0. 001 percent oil.

Precision in Replications by Multiple Operators

Only two laboratories reported variations greater than 0. 001 percent oil when a sample was tested by more than one operator. No explanation is available for the 0. 005 percent variation experienced by laboratory 4, as their tests were presumably limited to single strength or reconstituted juices. Laboratory 15, however, is connected with a plant that produces citrus oils, and it is probable that the samples which produced this result contained very high proportions of oil. Their report bore the remark that they had found it necessary to exercise extreme care in order to obtain a representative sample. Similar remarks were made by two other respondents.

Difficulties Encountered

Difficulty in observing the methyl orange endpoint and erratic results have already been mentioned. We believe this problem can be solved by maintaining optimum ratios between water, alcohol, acid, and indicator. Our experience indicates that alcohol content should be maintained between 40 and 70 percent, with a maximum solution volume of 60-ml.

Another laboratory reported breakage of distilling flasks due to their having boiled dry while unattended. Tests were conducted in that laboratory with additional water, and it was found that up to 25-ml. did not affect results.

One laboratory reported that, when the sample contains appreciable solids, as in centrifuge cream or extractor peel grit, a single distillation does not recover all the oil. Two or three successive additions of 2-propanol to the sample in the distilling flask resulted in successful recovery of all the oil, whereas doubling or tripling the alcohol content for a single distillation did not.

Summary and Conclusions

The preponderance of evidence to date indicates that there is no factorial relationship between oil recoveries by the Clevenger and the bromate methods. Although one careful operator might establish an additive relationship, the correlation would not apply among multiple operators. Reports from 15 laboratories indicate that the method is inherently precise and accurate, and is rapid and convenient to use. Principal precautions to be observed are (1) the small size of sample greatly increases the importance of exercising extreme care to make sure that the 25-ml. sample is representative, and (2) that proper ratios between alcohol, acid, water, indicator, and d-limonene be maintained by limiting the volume of distillate plus titrant to about 60-ml. (40-70 percent alcohol).

It is planned that sufficient additional data will be obtained during the coming season, by formal collaboration of several laboratories, to justify requesting the Association of Official Analytical Chemists to adopt the bromate method as an Official Method for the determination of recoverable oil in citrus juice products.

DISCUSSION

Question: Have you tested for normality the bromate solutions being supplied by chemical supply houses?

W. C. Scott: No, I have not. We assume that tests reported during our survey were made with commercially procured standard solutions. Since results were very good, I think we have good evidence that suppliers are delivering quality merchandise. We recommend purchase of 0.099N bromate, because if we ask for 0.1N the supplier will naturally make it accurate only to the second decimal place. When you specify third decimal accuracy, he will make it as accurately as possible.

Question: Have you done any work on adding known amounts of grapefruit, lemon, lime, and tangerine oils and determining absolute recovery, rather than as compared to recoveries by the Clevenger method?

W. C. Scott: No. The method was developed to determine recoverable oil, and its accuracy was checked by the percent recovery of freshly distilled oil, which is mostly d-limonene regardless of the fruit it came from. Since lemon oil contains larger quantities of α-pinene and citral than other citrus oils, I did distill some and study its recovery by the bromate method. I do not recall the results, but they were included in the data published. I have compared recoveries by the Clevenger and bromate methods of oils from grapefruit, tangerine, lemon and lime juices and purees. The bromate method showed recoveries equivalent to or slightly higher than those by the Clevenger, so we considered it to have sufficient accuracy to use in measuring recoverable oil from these products.

Question: I am not questioning the accuracy of the method. I am questioning the factor to be used to determine with accuracy the amounts of different oils, such as the amount of oil in lemons which would be different from the amount in oranges, even if they showed the same percent recoverable oil.

W. C. Scott: I'm sorry, we have not attempted to develop factors to convert recoverable oil to total oil. We feel that, if we did establish such factors, they would not be universally applicable.

FLAVORING EFFECTS OF NOOTKATONE IN GRAPEFRUIT JUICE

by

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(Presented by M. G. Moshonas)

During the investigation of the composition of the various citrus oils, a primary question in the mind of the research chemist is "What compound or group of compounds are responsible for the flavor and aroma of each oil?" Good answers have been presented for lemon and lime, and work is proceeding to find the answers for orange and tangerine. Today, I would like to talk about a sesquiterpene ketone named nootkatone, the one constituent found in both grapefruit peel oil and peel oil free grapefruit juice that has been shown to be a primary grapefruit flavor agent. More specifically, I would like to discuss the "flavoring effects of nootkatone in grapefruit juice."

Allow me to briefly summarize the endeavors of past researchers. Nootkatone was originally characterized as a heartwood constituent of Alaskan yellow cedar by Erdtman and Hirose in 1962. In 1964, MacLeod and Buigues of the USDA Laboratory in Pasadena reported nootkatone to be a constituent in grapefruit peel oil and juice.. stating at that time that "the flavor intensity of grapefruit oil appears to be related to the relative abundance of nootkatone." In 1965 Dr. MacLeod published the structure of nootkatone which is shown in figure 1. Also in 1965 Dr. G. L. K. Hunter and Mr. W. B. Brogden, Jr., reporting from our Laboratory, presented the synthesis of nootkatone from valencene, a sesquiterpene hydrocarbon isolated from orange oil. This reaction is shown in figure 2.

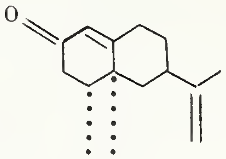

Figure 1.--Structure of nootkatone.
MacLeod, W.D., Jr., Tetrahedron Letters, 52, 4779(1965).

Although correlations have been observed between nootkatone content and general quality of grapefruit peel oil, the actual influence of nootkatone on taste of grapefruit products in a normally consumed form had not been established. Its taste threshold was determined to be from 20 to 40 p. p. m. in 32° Baume´ aqueous sucrose or 60 Brix, by MacLeod and Buigues in 1964. It would not however, necessarily be the same in single strength grapefruit juice. When synthetic nootkatone was added to juice prepared from reconstituted foam-mat dried grapefruit crystals, and this was tested in our taste lab., it became apparent that the level of 20 to 40 p. p. m. gave the juice a bitter taste and appeared far above the threshold. We then began to wonder whether this was due to trace impurities in the synthesized nootkatone or to the flavor of nootkatone itself. Since no threshold data were available on the relative effects of nootkatone on flavor of single strength grapefruit juice as normally consumed our laboratory made a study to establish this taste threshold, as well as, to

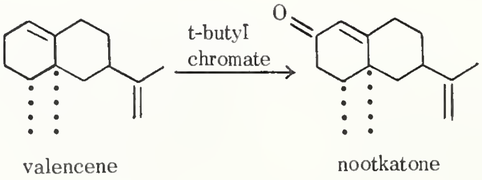

Figure 2.--Conversion of valencene to nootkatone.
Hunter, G.L.K. & Brogden, W.B., Jr., J. Food Sci. 30, 876(1965).

determine at what level the presence of nootkatone would be beneficial.

In order to provide information on the relative influence of nootkatone on the flavor and aroma of single strength juice, as well as the possible interference of any impurities in synthetic nootkatone, four samples of nootkatone were used. These were: (1) natural nootkatone separated from grapefruit peel oil; (2) synthetic nootkatone purified once by GLC; (3) synthetic nootkatone with repeated GLC purification, and (4) crystalline nootkatone.

To obtain the natural nootkatone, Florida cold-pressed grapefruit oil was distilled on an Arthur F. Smith Rota-Film Molecular still at 1 mm. Hg pressure at 80° C. to remove the large quantity of limonene. The nootkatone remained in the residue. This residue was redistilled in a Nester Faust spinning band semi-micro column and the fraction collected at 120° - 150° C. at 1 mm. Hg pressure contained the nootkatone. The nootkatone was made chromatographically pure by GLC.

The synthetic nootkatone was made from valencene which had been obtained from cold-pressed Florida orange peel oil. The valencene was oxidized to nootkatone by the method described by Hunter and Brogden. One sample of this synthetic nootkatone was purified by a single chromatographing which will be referred to as normal purification.

Another sample was chromatographed repeatedly by two different columns until there were no further detectable trace impurities, and will be referred to as purified synthetic nootkatone.

The fourth and final sample of nootkatone to be tested was chromatographically purified and then crystallized twice from cold hexane as described by MacLeod and Buigues. This yielded white crystals of nootkatone with a melting point of 35° to 36° C. Identifications were made by infrared and mass spectroscopy.

After numerous attempts at uniform distribution of nootkatone in an aqueous medium were made, excellent results were obtained when the nootkatone was dissolved in a minimum of alcohol and this solution diluted with glycerine to form a stock solution. For a stock solution, 25 μl. of nootkatone were dissolved in a 3 ml. of 95 percent ethanol. This ethanol solution was then dissolved in 25 ml. of glycerine. This gave a uniform dispersion which when diluted for taste studies did not contain enough ethanol to interfere.

Usually taste samples were made up in 1,200-ml. batches. To the experimental batch was added the required amount of nootkatone stock solution by injecting it from a micro syringe into the grapefruit crystals in the bottom of a beaker. Cold water was then added to the crystals and they were dissolved by stirring. Control batches were made in a similar manner without nootkatone. There was never more than 1° F. difference between samples when they were presented to the tasters.

All taste evaluations were made with the triangle or paired comparison tests. For triangle taste tests, 12 experienced tasters were each given two presentations and asked to indicate which sample was different. When the triangle tests were used, however, many of the tasters reported that there was a lingering bitter aftertaste which made evaluation of more than two samples very difficult. Therefore, most evaluations with juice were carried out by paired comparison tests. For paired comparisons tests, either 15 tasters were presented single pairs, or 13 tasters were each given two pairs and asked which sample they preferred, or which sample they thought contained nootkatone, or which was more bitter. These tests were also found to be more reliable when small pieces of unsalted soda crackers were provided for the tasters to chew between samples.

Tests were designed to determine the threshold level of nootkatone in distilled water and in 10.5° Brix grapefruit juice reconstituted from foam-mat dried grapefruit crystals with and without "locked-in" grapefruit peel oil. Several tests were used to determine whether a bitterness was attributable to nootkatone at certain levels.

Before studies were made of the threshold of nootkatone in juice a base threshold in water was desired. For the determination of the threshold in water, tests were made at a level which would normally be expected in grapefruit juice and the results are shown in table 1. This table shows that the taste threshold of nootkatone in distilled water is 1 p. p. m. at a confidence level of 1 percent for natural and synthetic pure and 1 p. p. m. at confidence level of 0. 1 percent synthetic normal.

Table 1. --Taste thresholds of nootkatone in pure water using triangle taste tests

Sample	Level p. p. m.	Confidence level %
Natural	0. 5	Not significant
Natural	1. 0	1
Natural	3. 0	1
Synthetic pure	1. 0	1
Synthetic normal	1. 0	0. 1

Taste thresholds were then studied in single-strength grapefruit juice containing oil. Analysis indicate that the base level of the nootkatone already in the juice crystals, plus that in the "locked-in" oil to be 0. 3 to 0. 5 p. p. m. This is still below the threshold in water. Results of this study are shown in table 2. Data reported here refer to amounts of added nootkatone above this base. Thus, it appears that the lowest detectable concentration is five to six times higher in juice than in pure water. The nootkatone crystals had the same threshold as the other samples indicating trace impurities in the other samples were of little influence on flavor. When this test was repeated in juice reconstituted from foam-mat dried crystals without added oil, a reduction of about 1 p. p. m. was found in the threshold, as will be seen in table 3.

During the testing of these samples there were several comments from tasters concerning the lingering bitterness they observed, especially in samples of higher nootkatone content. In a test to determine the relative influence of nootkatone on bitterness, three levels above the threshold were compared in juice from crystals without oil. Samples containing 6 p. p. m. added nootkatone were compared to samples containing 9 p. p. m. added nootkatone for preference and degree of bitterness by paired comparison. There was no significant difference. As a final study of this question, samples of 6 p. p. m. added nootkatone were compared to controls containing no added nootkatone and tasters were asked to judge the pair according to bitterness. The sample containing the added nootkatone was judged more bitter at the 1. 0 percent confidence level.

In a final study to determine the most desirable level of nootkatone, it was found that samples of 6 or 7 p. p. m. nootkatone were preferred when compared to controls with no added nootkatone. These samples were judged to have better aroma and fuller flavor.

The conclusions of this study were that nootkatone has a taste threshold of about 1 p. p. m. in distilled water and about 5 or 6 p. p. m. in grapefruit juice. Near threshold level, nootkatone contributes to grapefruit aroma and flavor and was found beneficial. However, if the level was increased much above 8 p. p. m. an unpleasant taste was encountered which was noticed by most tasters.

DISCUSSION

Question: Did you find great differences between people in the taste threshold level for nootkatone?

M. G. Moshonas: No, there were no great differences.

Table 2. --Taste threshold of nootkatone in single strength grapefruit juice reconstituted from foam-mat dried crystals with 0. 005 percent "locked-in" oil

Sample	Level p. p. m.	Confidence level percent	Type test
Natural	4	Not significant	Triangle
Natural	4	5	Pair
Natural	6	1	Pair
Synthetic pure	5	Not significant	Pair
Synthetic pure	6	5	Pair
Synthetic pure	5	Not significant	Pair
Synthetic normal	6	1	Pair
Crystals	5	Not significant	Pair
Crystals	6	1	Pair

Table 3.--Taste threshold of nootkatone in single strength juice reconstituted from foam-mat dried crystals without added oil

Sample	Level p. p. m.	Confidence level percent	Type test
Natural	3	Not significant	Triangle
Natural	4	Not significant	Triangle
Natural	4	1	Pair
Natural	5	1	Pair
Synthetic pure	4	Not significant	Pair
Synthetic pure	5	1	Pair
Synthetic normal	4	Not significant	Pair
Synthetic normal	5	1	Pair

A REVIEW OF FREEZE-DRYING STUDIES AT WINTER HAVEN ON CITRUS PRODUCTS

by
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(Presented by R. E. Berry)

During the past year a project has been originated which has as its objective the study of the freeze-drying characteristics of subtropical fruits. The project is broadly organized around two general areas. These are (1) fundamental investigations: determining the basic physics and chemistry of the freeze-drying process, and (2) applied investigations: the development of conditions for the optimum dehydration of products by the freeze-drying technique.

In order to be able to study freeze-drying of citrus fruits, and other products efficiently, freezing point data were needed. The freezing phase is considered by many to be as important as drying for the preparation of products of high quality. For example, products such as meats or fruit juices must be properly frozen before drying in order to retain desired organoleptic properties after rehydration.

In a number of previously published works it has been shown that freeze drying is most efficient, and the products are of highest quality when the product is not allowed to rise above its incipient melting point, until practically all of the water has been removed. It has also been shown previously that as a material becomes more solidly frozen, the electrical resistance of that material increases at an increasing rate. Thus, as a beginning stage of the study of freeze-drying, we began to measure the degree of solidification at different temperatures in several different types of food materials, using a measure of electrical resistance and particularly the rate of change in resistance as a measure of solidification.

These freezing points and electrical resistance measurements were made on a number of materials with very different characteristics. These ranged from aqueous extracts, such as coffee, through aqueous mixtures of soluble materials with dispersed insoluble solids, as represented by citrus juices, and also included vegetable cellular material consisting of ground celery and animal cellular material consisting of meats.

The apparatus used for these freezing point determinations consisted of a mechanically stirred insulated freezing dry-ice/alcohol bath, into which was immersed four electrically insulated 38- by 200-mm. Pyrex test tubes which were used as the freezing cells. Each cell had immersed in it two stainless steel 28-mm. wide and 44-mm. high electrodes on long rubber-insulated leads. The electrodes were curved to fit just inside the perimeter of the tube. Insulated stainless steel leads were used to connect these cells to the measuring instrument.

There has been considerable question in previous studies about the influence of polarizing voltages and currents when very high voltages are used at low temperatures, particularly in solutions of high electrolyte content. For this reason we studied these freezing point resistances with both direct current where polarizing voltages might exist, as well as with alternating current, where they should not. For direct current an Eico model 956 bridge analyzer was used as a measuring device. With alternating current an AC meter was used. Alternating current was provided by passing 60-cycle, 117-volt line voltage through a step-up transformer and a variable rheostat. Variable voltages up to 500 volts were applied across the electrodes in the freezing cell and the current passing across the electrodes was measured over the desired temperature range. From the voltage and the measured current the resistance of the sample was calculated by Ohm's law.

The samples were lowered in temperature until the resistance was higher than 500 megohms. Temperature was then increased slowly and change in resistance with change in temperature was plotted graphically.

As expected, the resistance of all samples increased markedly with lower temperatures. Single strength coffee and single strength orange juice had the higher resistances at higher temperatures (f. p. =-27° C. and -34° C., respectively). Most meat samples required lower temperatures for the higher resistances (ranging from -50° to -60°). It was also noted that the greater rate of change, that is, the greatest change in slope of the curve, appeared at approximately the 100 megohm resistance point with nearly all samples. Also it was noted that as the resistance approached the 500-megohm point the resistance rapidly approached infinity. Thus, not knowing what specific resistance value might be of principal influence, we made note particularly of the 100- and 500-megohm points for a number of samples in order to be better able to compare their electrical activity under these conditions. There were only a few degrees difference in temperature between these points.

Generally, the more complex a sample was the lower its freezing point. For example, the meat samples were probably among the more complex materials studied, and they required the lowest temperatures to reach the resistances of 100 or 500 megohms (in the range of -50° to -60°). Of course, none of these samples could be considered simple, but probably the citrus juices are simpler substances than the meats, and coffee is probably among the simplest of the samples, being mainly dissolved and dispersed colloidal materials. Citrus juices ranged from -35(11° Brix) to -48° C. (57° Brix) coffee ranged from -30°(2. 5 percent sol'n) to -42° C. (20 percent sol'n). Among any given material the higher the concentration the lower the freezing point. Orange concentrate, for example, required -40° and -45° C. respectively, for the 100- and 500-megohm points for a 57° Brix concentrate, whereas the corresponding temperatures were -38° and -42° for 30° Brix concentrate, and -35° and -39° C. for 11° Brix juice. Similar results are noted with grapefruit juice and coffee.

When similar data were obtained on these materials using alternating current instead of direct current, a number of similarities were observed, as well as certain differences. The curves were very similar in shape to those obtained using direct current. However, the values, particularly of resistance, were considerably lower. Whereas, with DC the principal break in the curve was around 100 megohms, with AC the principal break occurred around 1 to 2 megohms. Whereas, the region where resistance approaches infinity occurred around 500 megohms with DC, with AC it occurred around 10 megohms.

Using this region, around the 10 megohm resistance point, for comparison of different products, we saw that the relative positions of these different products were about the same as observed previously with DC. Single strength coffee (at about -23° C.) was followed by ss orange juice (=-30°), then 22° Brix orange concentrate (=-32°), ss lime juice (=-34°), then the different meat samples, (-35° to -45°) with cooked beef requiring the lowest temperatures of all.

The temperature points of the maximum break in the curves, that is, the maximum rate of change of resistance with change in temperature, were about the same with both the AC and the DC methods, particularly for citrus products. This was =-25° C. for ss coffee, -30° for ss orange juice, and -34° for ss lime juice. With meat samples, however, there was around 5° to 10° C. difference in the temperatures corresponding to respective areas of the curves. By DC measurements temperature requirements for meat samples were lower by 5° to 10° C., than those indicated during AC measurements (range of -55° to -60° with DC as opposed to -45° to -50° with AC).

A few applied studies have also been carried out on freeze drying during this time. Although we have not had sufficient experience to attempt to optimize conditions for any product, these precursory runs have shown some of the problems that need consideration and have given insight into techniques and procedures.

Conditions of drying for two noncitrus products were studied. Celery in 1/4-inch slices was dried at a maximum platen temperature of 135° F. and after drying time of 32 hours produced a product with very good flavor but with a fairly poor and prolonged reconstitution time. Whole cooked shrimp was dried at a temperature of 85° F. for 25 hours and a good quality product with moisture content of 2.5 percent was produced. In the first case, work is needed on reconstitution and in the second case on reduction of final product moisture. In both cases, it would be desirable to develop a system requiring shorter drying time.

A few preliminary drying runs have also been made on citrus products. These have included citrus sections and single-strength orange juice. We have produced dehydrated sections from temple oranges, tangerines, and grapefruit. They have all had good flavor but fairly high moisture content, and relatively poor reconstitution rates. The grapefruit sections were most difficult to reconstitute. In the production of freeze-dried orange juice we have conducted two preliminary experiments. In one we compared low (=1-1/2 lb./ft.^{2}) to higher (=3 lb./ft.^{2}) dryer load and in the other we compared two different product forms, one ground to a very fine powder (less than 20 mesh) and the other to coarse chunks. When low dryer load was used a very good product, with good reconstitution and 1.5 percent moisture content was produced. A similar run using twice the load required almost twice as long to dry and had a much higher moisture content with a number of wet spots in the trays. In the comparison of different grinds, it was found that the fine grind product dried to a lower moisture content than the coarse grind product, under identical conditions. This would imply that residual moisture content, and possibly drying time, could be to some extent controlled by the original form of the initial product.

To summarize, most of our work on freeze-drying to date has been exploratory. It is obvious that very low temperatures will be required to bring about complete freezing before freeze-drying of most materials. A system based on measure of electrical properties can be used for determination of freezing points. The system described here is simple, easy to operate, and would be easily set up in a plant. Freeze-drying rates and the quality of the final product are without question directly related to the form of the original material and to the conditions of drying. A number of fairly good products have been produced by freeze-drying, but conditions that are optimum for drying rates and product quality have not been developed. No information is available as yet concerning the storage stability of these products and its relationship to drying conditions.

DISCUSSION

Question: Indications by your curves were that a lot of these "frozen foods" are not frozen?

R. E. Berry: That is the way it looks. From the curves we obtained it would appear that some of the previously reported experiments on freeze-drying have possibly been conducted with materials that were not completely frozen.

RECOVERY OF PIGMENTS FROM ORANGE PEEL AND USE IN ENHANCING COLOR OF ORANGE JUICE

by
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(Presented by J. F. Fisher)

This paper is presented as a progress report and should not be considered as a completed piece of work.

In view of the Florida regulations concerning the addition of noncitrus ingredients to citrus products, we are investigating the possibility of extracting the orange coloring material from citrus peel relatively free of an undesirable taste. This colored citrus material might then be used to enhance the appearance of pale orange juice.

It should be pointed out that the extraction of orange color from orange peel is not a problem. However, obtaining this color free of unwanted taste is a problem. Most of the steps in the following tentative procedures are mainly directed at removing the unfavorable taste constituents.

A deep orange colored material relatively free of undesirable taste is obtained from good colored Valencia orange peel. This colored material is extracted, processed, and used to enhance the appearance of light colored orange juice by the following procedure.

1. Thirty pounds of good orange colored Valencia orange peel (these oranges were picked during the height of the Valencia season) is extracted with 8-10 liters of n-hexane in a Waring blendor at room temperature.

2. The extract is filtered and evaporated to an orange colored residue of approximately 100 ml.

3. This residue is chromatographed on a column of silica gel using n-hexane and 3 percent acetone-hexane for elution. An orange colored band which separated from the chlorophyll and yellow pigments is collected. The solvent from this orange colored fraction is removed and the residue rechromatographed on a column of magnesium oxide, again using hexane and 3 percent acetone-hexane.

4. The orange colored band is collected and the solvent removed in vacuo.

5. Water is added to the residue and the vacuum distillation at approximately 40° C. is contained until the added water is essentially removed.

6. If the consistency of the deep orange-colored residue is liquid enough, it is added dropwise with shaking to the pale juice. However, if the residue is too viscous for this it is dispersed in orange juice by mechanical agitation.

7. The color enhancement is determined with a MacBeth color comparator.

When we add the color enhancer dropwise, 1 to 4 drops is sufficient to raise 50 ml. of substandard juice of about 34 points by the MacBeth system to an acceptable 36 to 38 points.

The color-enhanced juice was tested in a taste panel, which was conducted in the following manner. The color-enhanced juice samples were distributed without a control sample. A reply to the question "What do you think of this juice" was requested; 88 out of 89 people responded favorably. Where the color-enhanced juice was subjected to a triangle taste test and the panel was asked to select the different sample, the color-enhanced juice was selected without difficulty. However, numerous participants remarked that while the colored juice tasted different it was not an objectionable taste difference.

The tentative procedure just outlined requires both simplification and refinements, but the procedure appears to work reasonably well if prime Valencia orange peel is employed. Shortly after this work was started we ran out of our good orange colored peel and were forced to use peel that had already entered the regreening process. With this peel the procedure afforded material with less color but, even more disturbing, the colored material had an undesirable taste.

In the regreening process the carotenoid synthesis declines while the chlorophyll synthesis increases. It may be that other, perhaps undesirable taste, constituents such as certain flavonoids also build up.

Lyle Swift of our Laboratory has shown that several of the aglycosidic flavonoids of orange peel have undesirable tastes. On the assumption that such compounds may be increasing in the regreening peel and carried through the procedure, we removed them by cooling the residue in step 2 and bubbling through it anhydrous hydrochloric acid which precipitates flavonoids and similarly oxygenated compounds as their oxonium salts. The mixture at this point is dark green. It is refrigerated at 40° F. for several hours and filtered. The filtrate is placed in vacuo to remove some dissolved hydrochloric acid and then swirled with a few pellets of sodium hydroxide, which returns the orange color.

While this procedure works well for removing flavonoids as their oxonium salts, it does not improve the taste of the color enhancer, neither is it detrimental to the taste. We have tentatively concluded that our taste problem with less orange colored peel is not due to flavonoids. This has been a tangent of interest rather than the main approach which of course is to extract good colored peel.

In lieu of good colored peel, we have tried pulp, but that which we have tried lacks sufficient color.

We have tangerine peel and are preparing to explore its use.

In summary we appear to have, on the basis of a few experiments, a tentative procedure which will extract from good colored orange peel a coloring material relatively free of an undesirable taste.

The quantity of color enhancer obtained from the peel in one box of fruit is estimated to be sufficient to raise 5 to 10 gallons of substandard juice to an acceptable color value.

DISCUSSION

Question: Have you tried recovering color from tangerines?

J. F. Fisher: No. We haven't tried anything but the peel of Valencia oranges as just described. We plan to extract tangerine peel, but have very little to report at this time.

Question: I assume you will work with some early highly colored fruit, say in January or February?

J. F. Fisher: Just as soon as we can get some good colored peel, whether it be pineapple, tangerine, or highly colored Valencia.

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